

## **The Chemical Industry comments on State-of-the-Art report on Mixture Toxicity**

The European chemical industry, here represented by their associations CEFIC and ECPA, as well as the scientific forum ECETOC, appreciate the opportunity to comment on the State-of-the-Art report on mixtures toxicity by Kortenkamp, Backhaus and Faust, contracted by the European Commission.

### **Overview**

We recognize the need to address scientific and public concerns about cumulative exposures and their potential adverse environment and health effects. The scientific complexity arising from the potential infinite array of possible chemical mixture combinations should be balanced with the real-life situations (i.e. realistic exposure scenarios), the objectives of the improvement of environment and health quality and the feasibility and practicality of regulatory actions.

First, given the above mentioned challenge and important consequences of future policy decisions, we strongly believe that it is fundamental to consider as much as possible the relevant available knowledge that allows for a robust scoping of the next steps. In addition, it is essential that conclusions of such a State-of-the-Art report should be supported by this relevant literature in order to be a robust basis for policy making. In that respect, the current report has serious shortcomings. In Annex I, we have compiled a series of additional studies (not considered in the report), pertinent to the issue at hand.

Second, we would like to make a preliminary remark on the definition of mixtures. As already specified by the official text accompanying the publication of this report (see DG ENV website), a broad definition, such as the one used in the study, makes a systematic, comprehensive and integrated approach / action unrealistic (including the aspect of animal testing).

Third, the generalization, in the report, that “Mixture Risk Assessment [RA] is necessary to avoid underestimation of risk” is not supported by adequate evidence. In specific cases, there may be potential value for a specific approach such as Mixture RA; current risk assessments on single substances are already based on several worst case assumptions and elements of precaution.

Finally, we strongly recommend that any program for the investigation of cumulative risks should be applied in a targeted manner. We believe that cumulative risk assessments can be used to assess the mixtures of greatest concern, and methodologies for the identification of such mixtures can be done by using approaches that are currently discussed e.g. within the chemicals industry Long Range research Initiative (LRI).

### **Conclusions**

The report is a starting point as it gives a first overview on this complex issue but further discussion is needed along the main lines outlined above. We would appreciate further opportunities to elaborate these comments and a future approach as preparatory work for the planned EC workshop in June 2010.

The following comments provide a more detailed evaluation of the content of the report, and substantiate the industry comments outlined above.

### **1. Report Statement: “Mixture RA is necessary to avoid underestimation of risk”**

**Industry comment:** This statement assumes that industry and regulatory risk assessors are currently underestimating risk with individual chemical-by-chemical assessments. As it will be detailed further on, there is little evidence to support this assertion. Current risk assessments on chemicals are highly conservative, giving confidence that additive or even rare synergistic effects are likely to be accounted for.

Current risk assessment procedures have been in place for many years and proved their value; before deconstructing, evidence of their shortcomings should first be documented. Occupational risks, for example, are now well established and occupational diseases/chemical accidents have decreased to low numbers. We welcome a review of risk assessment procedures but caution against a change without first demonstrating limitations in the current approach. Additional use of a ‘mixtures’ component should be on a well justified basis, using clear criteria, such as mode of action grouping, and with parameters that are embedded in recognized toxicological methodology for hazard identification and risk assessment.

From the environmental perspective, the report provides a good summary of the general principles and approaches for assessing the toxicity of chemical mixtures; this is something that has been done repeatedly since the 1980s with little change in the conclusions or recommendations. Where toxicity of mixtures needs to be evaluated for environmental risk assessment purposes, the model of concentration addition provides a conservative default approach as a first tier.

However, the report goes beyond mixture toxicity, into risk assessment and the environmental impact of mixtures. Here, it fails to substantiate the claims made in the Executive Summary *“There is a consensus in the field of mixture toxicology that the customary chemical-by-chemical approach to risk assessment might be too simplistic. It is in danger of underestimating the risk of chemicals to human health and to the environment”*. The document provides no evidence that the current risk assessment practices for chemicals fail to provide adequate protection (see Annex I).

### **2. Report Statement: “Mixture RA is feasible as demonstrated by practice in USA”**

**Industry comment:** We agree that the scientific state of the art mixture toxicology has been advanced during the last years, but we do not agree that it shows that mixture risk assessment in general and broadly in the EU is necessary and/or feasible. Currently generalisation is not possible and the real need for cumulative risk assessment (e.g. for “hot spots”) needs to be evaluated on a case-by-case basis. The process needs to be well-defined, scientifically sound, transparent, risk based (taking into consideration realistic exposure scenarios) and able to assure a consistent approach.

Some parts of US legislation do use approaches for evaluating the risks from mixtures of some chemical substances and have been captured within Part 4 of the report. The fact that there are approaches, which have been used in some specific cases, does not make their use justified as a default for all risk assessments. The use of an arbitrary assessment factor may be easy and pragmatic but has little technical validity other than increasing the conservatism which is already built into the use of assessment factors. Other approaches must be carefully reviewed and valued for their applicability before being adopted (see Annex I).

In summary, the report fails to explain what is involved in the various US guidance documents. It does not explain the important concepts created in each of the EPA guidance documents; e.g. as early as the 2000 supplementary guidance to the 1986 documents, concepts of internal dose, pharmacokinetics, PBPK modeling and pharmacodynamic modeling were mentioned. The 2003 and 2006 documents expand on these. Nowhere in the state-of-the-art report are they mentioned (see Annex I).

This leads to the conclusion that the presented data do not create a basis to decide whether or not US practices would be feasible.

### **3. Report Statements on scientific aspects:**

#### **a. “Significant number of decisive mixture studies were carried out”**

**Industry comment:** The authors do present a fair number of studies to assess mixture toxicity; both for mammalian toxicity and eco-toxicity endpoints. However, the report lacks transparency since it is unclear if a real State-of-the-Art review was carried out since the cited literature seems to be incomplete. In this respect, we have included additional citations (see Annex I).

The report claims to describe the state of the art in the field of mixture toxicity and also the regulatory state of the art for dealing with combined exposures.

Unfortunately, information in the form of systematic observations in human populations is lacking, and therefore, only animal hazard data forms the basis for experimental evidence supporting mixture toxicity. Regulatory agencies have expressed their view, that evidence from high quality, thoroughly conducted epidemiologic studies are critical in assessing evidence from animal models. When available, comparisons between human and animal data help reveal consistencies and strengthen evidence for or against the toxicity in question.

Also for environmental aspects, the report does not represent information necessary for a valued judgement. Largely missing are basics such as relationship of external concentration and internal dose or species sensitivity (see Annex I).

#### **b. “Mixture effects more pronounced than individual components”**

**Industry comment:** Mixture effects, if they do occur, may be also less pronounced? What if you have a mixture of an estrogenic compound and an anti-estrogenic compound? There are many biological processes where mixtures of chemicals may lead to lower response due to competing or compensatory events.

In the State-of-the-Art report, Ruediger (2006) is cited with the statement “...Rather, an inhibitory effect appears to be possible only if the suppressing component has a weaker carcinogenic potency than the other chemical in the combination.”

This is also supported by an un-cited publication by Bolt (2001) (see Annex I) where it is shown that it may not be a rare case, that in the light of potency-weighted exposures, a single compound, either of natural origin or man-made, clearly dominates the exposure scenario rendering a specific assessment for mixtures dispensable. In other cases, exposure levels of all compounds may be so low that no assessment of mixture effects is necessary. Unfortunately, comprehensive exposure assessments that would allow in depth evaluation of these concepts are not available.

The major issue with mixtures in the environment is not their toxicity, but the exposure itself. Toxicity data to date indicates that the toxicity of simple and complex mixtures can be predicted. In the case of exposure, modeled exposures need to be derived, which reflect realistic environmental exposures temporally and spatially rather than multiple worst-case estimates (an example is given in Annex II of our comments). Environmental exposures to chemicals generally will be highly variable and difficult to predict. Nevertheless, in practice, certainly at the edge of field where pesticides are evaluated, exposures are more predictable and the majority of toxicity observed is likely to be explained by the most toxic component - a view supported by the toxicity testing of mixture formulations. Thus a combination of single compound testing and evaluation, together with limited toxicity testing of mixture products will serve to protect the environment.

Similar findings occur at hazardous waste sites. As discussed by the authors, the U.S. Superfund program has been assessing cumulative risks for more than 20 years. Under this program, waste sites go through a process where samples of air, water, soil, and waste materials are analyzed for a wide range of chemicals of concern. As a result, most sites have determined that a large number of chemicals of concern are present. The number of chemicals of concern for larger sites can exceed fifty compounds. However for the majority of sites, the risks to an exposed population (exposed by a specific pathway) have almost always be driven by one compound.

The fact that real world mixtures tend to be dominated by the toxic effects of one chemical is not widely recognized by researchers. Toxicologists generally study mixtures that have been intentionally designed to optimize the chance for mixture effects. As discussed in (USEPA, 2000), the difference between the response from an additive and independence model of toxicity is greatest when the two components are equipotent. For example it is common practice to test mixtures of “n” components using mixtures where each component is given at a dose equal to or just below the component’s no effect level or where the dose “1/n” of the component’s no effect level. These mixtures can be thought of a being on the midpoint of the isobolographs. The design of these mixtures is driven by the goal to optimize the ability to measure the differences between dose addition and dose independence (see tables 6.1 to 6.3 in section 1 of the report for examples of such mixtures). The authors’ implicit assumption that the intentionally designed mixtures tested in the laboratory are reasonable representations of real world mixtures is perhaps one of the largest limitations of the report.

This conclusion has been supported by recent research that has shown that the toxicity of real world the mixtures tends to be dominated by just one or two chemicals in the mixture (Price and Wiltshire, 2009; Price et al., 2009). Thus, reducing exposure to prevent doses of the most toxic component from exceeding its chronic standard will result in all other components being kept to doses that are well below their respective chronic standards. When a mixture is dominated by a single compound, the finding of risk from a cumulative assessment and a chemical-by-chemical assessment will produce the same estimate of risk.

### **c. “Single chemical RA in danger to underestimate risk”**

**Industry comment:** There is no evidence for this statement within the report. The practice is a “realistic worst case” such that emissions are estimated as being high compared to a low extrapolation from NOAEL levels to a highly conservative assessment. For intended or known joint emissions, mixtures toxicity can be relevant.

Single chemical RA should still be the preferred model and use of mixture interactions only be applied on a specific needs basis. Such needs must be based on consistent criteria such as dose-response effect for key toxicological finding(s), mode of action, exposure estimations, metabolic interdependencies, species sensitivity etc.

The current system of regulating individual chemicals, while not intentionally designed to address cumulative effects, has nevertheless established approaches that will accommodate risks from mixtures. In section 7.1 of part 1 of the report the authors correctly observe that none of the safety factors currently used are intended to address mixture effects.

Finally, the authors incorrectly state that safety factors cannot be equated with probabilities and that a direct translation “is not possible”. As discussed by multiple research over the last 15 years, it is possible to define the probability that the adjustment necessary for converting an animal POD to a chronic standard protective of a sensitive human requires a specific sized safety factor (Baird et al., 1996; Price et al., 2009; Schneider et al., 2006; Slob and Pieters, 1998; Swartout et al., 1998; Vermeire et al., 1991).

**d. “Environmental pollution by mixtures rather than individual substances”**

**Industry comment:** Methods such as direct toxicity assessment are reviewed in the report and the general view is that mixture assessment in the environment is more acceptable than for human safety. However, risk management to mitigate any effects will usually have to address the component activities of the mixture.

**e. “Concentration or dose addition = way forward”**

**Industry comment:** The assumption that dose addition when observed at high doses will also be observed at low doses is false. Dose addition (non-independent action) may occur at high doses while response addition (independent action) occurs at low doses for some groups of chemicals. As stated by Borgert et al., 2004, dose addition may be a conservative assumption [for some effects] of chemicals when they are present at concentrations above their NOAELs, but that independence becomes more predictive when the concentrations of the component chemicals are below their individual NOAELs.

It is important to point out that the reason low dose mixtures may be less than additive is that the mode of action could be different and would thus be below the NOAEL.

Borgert et al., 2004 also indicates that it is premature to assume dose addition for chemicals that appear to be mechanistically similar and to assume response addition models only for chemicals that appear to be mechanistically dissimilar. Because these simple models were developed for binary mixtures, their applicability to more complex mixtures is uncertain. Dose addition should be correlated with specific mechanistic features for particular toxic effects before the approach is generalized.

Whereas it is plausible that at doses near the NOAEL dose addition can occur, this is not true for very low doses. This is confirmed by the authors of the report (see e.g. 1.8) as well as of often-cited studies (e.g. Christiansen et al. 2009) and therefore should be considered in the conclusions of the report. “High dose mixtures” clearly need to be separated from mixtures containing very low doses.

#### f. “Better to take benchmark than NOAEL”

**Industry comment:** The use of benchmark dose instead of NOAEL is not currently feasible and this situation will not change in the near future, as the most important and regulatory relevant studies are done under OECD test guidelines and (in the area of human health) typically use 3 dose groups. A change in the paradigm of dose selection would influence the test guidelines and existing regulatory testing strategies; the risks and benefits from such a change need to be evaluated before a decision could be taken. Regulations such as REACH inherently use a PNEC / DNEL approach which requires a minimum of a NOAEL to identify the point of departure.

It is not clear why the author ties up the issue of BMDs with mixture toxicology since it is clearly a separate issue, which can be considered as a refinement of chemical risk assessment on a case by case basis.

#### g. “Research is needed for synergistic effects“

**Industry comment:** It is not clear from the report what research is needed to make a decision on whether mixtures need to be considered as a separate item and also whether additivity should be the default assumption. Case-by-case assessments would be a better starting point where data could be generated to address specific classes of chemicals rather than making general (policy) decisions on a restricted set of data.

Research has already been done with results being largely negative in terms of synergistic effects (only a few interesting examples were positive). It seems that the authors are strong believers that all mixtures produce some combination effects. However such effects cannot be assumed but need to be considered in view of all evidence.

#### h. “Low dose effects”

**Industry comment:** The authors clearly expects no effects from mixtures to be detectable when tested at low (i.e. relevant) doses (2<sup>nd</sup> from last para and last para of p134), and we agree. The report states “It would be trivial to attempt and experiment where for example two agents are combined at 1/100<sup>th</sup> of their individual NOAEL resulting in mixture effects, although existing, would be too small to be detectable.”

Throughout the report the term low dose is used to describe doses close to the NOAEL (see titles of sections 6, 6.2, 6.7). Care should be taken to avoid such confusion as although it is clear that multiple chemicals at or close to their NOAELs in a mixture can result in an effect, this has not been demonstrated for mixtures of components at or close to their reference doses.

Low doses are doses around or below the Reference Dose (Reference dose = NOAEL divided by the Safety Factor). These are actionable levels for regulated chemicals.

In addition, many of the key laboratory studies cited in EU State-of-the-Art report assessed mixtures in which the dose of each chemical was at near or above its threshold for toxicity. Therefore it should come as no surprise that combined effects were observed. In section 6 of Part 1, the report asserts that additivity also occurs at low doses.

Human exposures to man-made chemicals at or around the rodent NOAEL are not permitted under existing chemical risk assessment methodologies, and such exposures represent a frank failure of regulation on the occasions when they occurs. This being the case, if it is artificial mixtures that are to be tested then the most decisive mixture studies to conduct would be those where the individual components were present around the permitted reference doses. Unfortunately, only a few studies investigating mixtures at relevant dose levels (e.g. ADI)

were quoted in the report and these were by and large dismissed on the basis that the doses used were too low and/or the study design was inappropriate/insufficiently robust (p144-145 of Part 1).

Finally, the evidence for ‘low dose effects’ of endocrine disruptors comes mostly from *in vitro* approaches and this is not reflected in the report’s executive summary. The report treats these with an equal weight to the data derived from *in vivo* studies where biological adaptation of complexity of interrelated processes can be taken into account. Yet no consideration or comment appears to be given to the relevance of doses tested in these types of studies to potential human exposures.

#### 4. Report statements on Regulatory approaches

##### a. “Future EU guideline to go beyond existing regulatory approaches “

**Industry comment:** Substances should continue to be assessed individually to ensure all potential hazards are understood and to develop dose-response information to manage risks. Classes of chemicals should not be assessed simply on structural similarity unless there is sound rationale for read-across / category approaches. Where exposure information suggests possible combined exposures, it may be feasible to examine interactions of a substance within the context of its own risk assessment and to take into account structurally / mechanistically similar chemicals. Methodology to handle this is noted in the report, but no single approach is proposed. Any such approach requires a full study using either actual or derived data to review the consequences of applying mixture methodology.

As stated earlier, we believe that cumulative risk assessments should be applied in a targeted manner, rather than as a general matter of policy. Current risk assessment methods based on determination of Points of Departure (POD) and application of uncertainty factors should be protective for the vast majority of typical low level mixture exposures. This has been shown empirically in a large number of studies. Also industry has developed some ideas on the basis of LRI activities and would be happy to discuss.

##### b. “Focus should be on assessing and controlling exposure”

**Industry comment:** We agree that more information is needed on relevant exposures with respect to chemical mixtures. In addition, such exposures must also take into consideration naturally occurring substances.

Several authorities within the European community already assess and control environmental mixtures e.g. for plant protection products; however it should be noted that in such cases whole mixtures rather than component based mixtures are generally investigated (see Annex I).

##### c. “RA Uncertainty factor of 100 enough for mixtures?”

**Industry comment:** This seems to be a huge overestimation of the types of defaults that could be incorporated to account for mixture effects. No evidence is provided to substantiate this claim. The authors also state multiple times that dose-addition should be utilized in practice which in and of itself is conservative in nature.

Extra uncertainty factors (UFs) should not be considered a default practice, especially when considered on top of the considerable number of default AS already applied in REACH for chemical registration. They should be used in exceptional cases and reflect the exposure

profile, as well as the level of concern on the nature of the identified hazard. We support the ECETOC report on UFs (see Annex I) where their use is thoroughly reviewed.

**d. “Tiered approaches to RA of chemical mixtures needed”**

**Industry comment:** A tiered approach to conducting cumulative risk assessment based on the database for a group of chemicals seems to be a practical way forward. This is to start with simple yet highly conservative dose-addition and moving on to more data intensive methods as needs progress and / or change. The authors, however, provide no examples or proposals as to what more data intensive methods may be useful.

**e. “Cumulative RA”**

**Industry comment:** Real cumulative risk assessment will be impractical across different chemical types and dissimilar chemicals. For example, all kidney toxicants being assessed in a single group, from pesticides to industrial chemicals, and logically also to pharmaceuticals, food additives, naturally occurring food components, etc.

Before mixture risk assessment can be considered feasible, the manner in which chemicals should be grouped so as to be considered as a relevant mixture must first be addressed. Often this grouping is based on similar modes of actions but there is, as yet, no clear guidance on what could be considered as similar or dissimilar.

## Annex I

### **Some additional pertinent literature (not cited in the report)**

#### **Method/Review:**

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## Regulation

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## Annex II

In experiments that are carried out to demonstrate the effects of mixtures the concentration of each component are usually selected in a way, that the toxicity of each component contributes to the overall effect (e.g. for each component the EC<sub>20</sub> is applied, which leads to a measurable additive effect). However, under real environmental conditions this situation is very unlikely, since the concentration in a real mixture is determined not by its toxicity, but by other factors.

This is demonstrated by a theoretical example for an aquatic toxicity test:

Component A: EC<sub>50</sub> = 1 mg/L

Component B: EC<sub>50</sub> = 0.01 mg/L

Typical experimental situation: Tested mixture is composed of 99 g A + 1 g B. 50% of the effect is observed with a mixture of 0.5 mg A/L and 0.005 mg B/L.

Typical exposure situation: A and B are mixed e.g. in equal parts. The EC50 of the mixture is reached when the concentration of B is 0.01 mg/L. The component A (concentration also 0.01 mg/L) has no relevant influence on the overall toxicity, since it is a factor of 100 below its own EC50.